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I. V79 INHIBITION OF METABOLIC COOPERATION (IMC) ASSAY

With the expert assistance of Mr. John Tindall of the Product Evaluation Division, a method was developed for determining specific activity (percent recovery of 6-thioguanine resistant cells per ug/ml of test substance) (1). The IMC data best fit various types of a linear mathematical model so that the slope is an accurate representation of specific activity. Table 1 gives the specific activity of a variety of agents tested in the IMC assay. The larger the number the more active the agent.

Table 1. Specific Activity of Several Agents Tested in the IMC Assay.

TEST AGENT	SLOPE (Specific Activity)*
12-O-tetradecanoyl-phorbol-13-acetate	46443
catechol	11
CSC from LTF-5E	2
CSC from 2R1	0.8
saccharin	0.002
phenol	not active

* Specific activity = percent recovery of 6-thioguanine resistant cells per ug/ml of test substance.

Mr. Tindall also described a statistical method for comparing the specific activities of two active agents to determine if they are differentially active. When this method (analysis of variance followed by separation of means) was applied to the agents listed in Table 1, each was separable from the other (differentially

active) at the 95% confidence level. These statistical techniques will be applied to other test substances when the data are available, keeping in mind that specific activity should also be determined using other units of measure (nanomoles, cigarette deliveries, etc.).

Laboratory work has been completed with cigarettes containing high and low alkaloid filler levels (2.75% versus 0.51%) (2). While these CSCs were active, they did not appear to be different from each other. Confirmation of this conclusion as well as the relationship of these CSCs to the agents given in Table 1 awaits the analysis of the data using the statistical techniques described above. These latter two statements were also true for the cigarette with high nitrate in the filler (0.91%) collected by two different condensate trapping methods (dry, impaction-trapped processed versus cold-trapped processed) (3). All of this effort is directed towards attempting to understand what controls cigarette activity in this assay. CSCs from a 100% bright (2), a 100% burley (2) or a synthetic, nitrogen-free (LTF-2A) (3) filler are currently being tested to help answer this question.

Data have been generated and statistically analyzed which revealed that CSC obtained from synthetic, nitrogen-free filler (LTF-5E) can be stored (-80°C in dimethyl sulfoxide) for at least 2 months without losing any significant IMC activity (3).

Incorporation of an exogenous metabolic activation system with this bioassay has been hampered by the lack of success with obtaining a cofactor mix (as part of the metabolic activation system) that is compatible with the assay. We have determined that it is the energy source in the cofactor mix (the NADP or NAPDH) that in some way(s) renders the 6-thioguanine sensitive cells immune to killing by 6-thioguanine (3,4). This affords the sensitive cells a longer time to metabolically cooperate with resistant cells which finally results in a greater loss of resistant cells in the presence of the cofactor mix than in the absence of the cofactor mix. Currently we are attempting to determine if this "enhancement of metabolic cooperation" will be a problem when actual test agents are used in the presence of cofactor mix. Attempts will be made to alleviate the cofactor mix block by rinsing the plates after the cofactor mix treatment or increasing the concentration of 6-thioguanine in the treatment medium.

II. REFERENCES

1. McCuen, R. W. Notebook No. 8136; pp. 5-6.
2. Penn, J. M. Notebook No. 8104; pp. 71-72.
3. Ayers, D. J. Notebook No. 8107; pp. 105-109.
4. Garcia, H. D. Notebook No. 8051, p. 167.

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